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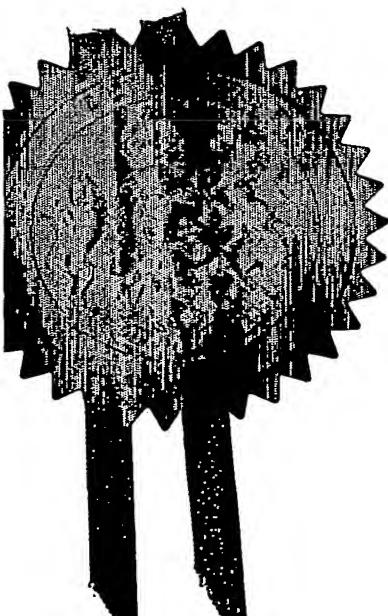
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"GROWTH SUBSTRATE"

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INTRODUCTION

Controlled Release Glass (CRG) is a phosphate based material which degrades at a predetermined rate. The potential for using CRG as a cartilage engineering matrix has been assessed using isolated equine chondrocytes with *in-vitro* techniques. The glass was provided in fibrous form in three different compositions by Giltech Ltd.. The three CRG compositions provided have showed potential as a tissue engineering substrate.

MATERIALS AND METHOD

A total of 200,000 chondrocytes isolated from horse articular cartilage were added to each 2cm well in a 24 well plate. Every well contained 0.02grams of glass fibre sample. Four different fibres (diameters 20-30 μ m) were analysed: F1 - containing Fe_2O_3 and NaF, F2 - containing Ce_2O_3 and F3 - containing Ce_2O_3 and Se. The culture medium (containing 10% FCS) was changed daily. At time periods of 3 days, 1 week and 2 weeks, the samples were stained using rhodamine phalloidin and oregon green for the viewing of actin and tubulin using a laser scanning confocal microscope. At the same time periods, the cell supernatant was removed and stored at -80°C until analysis on cell viability and type II collagen production could be performed. Production of type II collagen was analysed by using RT-PCR analysis on the cDNA from the chondrocyte population in contact with the glass fibres. The total RNA was prepared from the cell population by the addition of 1ml of TRIzol (SIGMA) to the cell population for 5 minutes. After this time, The TRIzol was retrieved and stored at -80°C until RT-PCR analysis could be carried out. The RT-PCR analysis was performed by tagging with primers for collagen type II and with gapDH for cell viability.

Zymography was also performed at time periods of 4 days, 1 week and 2 weeks for

detection of matrix metalloproteinases (MMP's) produced by the chondrocytes.

RESULTS AND CONCLUSIONS

Chondrocytes adhered to all three types of fibre sample. At the 3 day time period, the cells appeared to be rounded. At 1 week and 2 weeks, confocal microscopy indicated cell proliferation between all time periods. At 1 week and 2 weeks, the cells were elongated and formed a monolayer along the fibre length as can be seen in Figure 1.

The RT-PCR analysis showed that fibres F2 and F3 were producing collagen type II up to and including the two week time period indicating that the cells retained their chondrocytic phenotype.

The zymography performed on F2 and F3, showed that the cells in contact with these fibres produced MMP2 at all three time periods, but in a greater quantity at 2 weeks than 1 week, and at 1 week than 4 days. This increase of MMP2 production is expected, as the cells were seen to have increased in number at these time periods from the confocal microscope analysis.

In conclusion, all three fibres types showed cell adherence and the chondrocytes adhered to F2 and F3 appear to retain the ability to produce type II collagen.

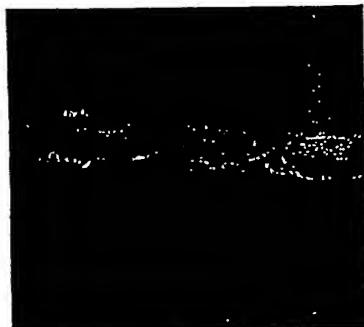


Figure 1. Adhered chondrocytes to fibre F1 at 2 weeks.

The authors wish to acknowledge the EPSRC for funding of this work.

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Figure 1. Adhered chondrocytes to fibre F1 at 2 weeks.

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